Annual gonadal cycle of the land snail *Scutalus tupacii* (Pulmonata: Bulimulidae)

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Abstract: The annual gonadal cycle and its relation to the seasons were investigated in the land snail *Scutalus tupacii* (d'Orbigny). Collections of adult snails were carried out monthly over two consecutive years. The animals were processed for anatomical and histological studies. The ovotestis is composed of a number of tubules or elongated acini, each one containing both male and female sexual cells as well as Sertoli and follicular cells. Spermatogenesis and oogenesis occur in all the acini.

The annual cycle of the ovotestis was divided into three phases: 1) Pre-breeding (September-November: Spring), characterized by the reactivation of spermiogenesis and the subsequent increase in number of mature sperm; 2) Breeding (November-February: Spring-Summer), characterized by the great number of spermatozoa in the gonad, and the highest proportions of mature oocytes; 3) Post Breeding (March -September: Autumn-Winter, with abundance of spermatogonia, oogonia and primary spermatocytes, and the interruption of spermiogenesis.

Although the proliferation of male and female germinal cells is practically coincident, male gametes attained maturity at least one month before the female gametes. The post-breeding period occurs during hibernation which is coincident with the dry season. Based on the data obtained, the annual cycle of reproductive activity in this species depends principally upon the wet or rainy season.

The annual gonadal cycle of many species has been previously investigated [e.g. *Lymnaea stagnalis* (Linné) (Berrie, 1966); *Helix pomatia* (Lind, 1973); *Semperula maculata* (Tompleton) (Nanaware and Varute, 1975); *Helix aspersa* Müller (Gomot and Griffond, 1987)]. Related works include research on the maturation of the reproductive tract of various species (see Lusis, 1966; Smith, 1966; Runham and Laryea, 1968; Sokolove and McCrone, 1978; Runham and Hogg, 1979; Cuezzo, 1990) as well as the life cycles of a number of groups (see Van Der Laan, 1975, 1980; Hodasi, 1979; Bailey, 1980; Solem, 1984; Roth, 1986). More recently, there have been a growing number of studies that analyse the influence of different factors on gametogenesis under laboratory conditions (Gomot and Gomot, 1988; Gomot, Gomot and Griffond, 1989; Griffond and Medina, 1989).

The genus *Scutalus* Albers, 1850, is well represented in the western region of Tucumán Province (27°S, 66°W), Argentina. Despite the large and widespread populations, very few data are available regarding the biology of these bulimulid land snails. In the hope of filling part of this gap, a population of *S. tupacii* (d'Orbigny) was sampled over two consecutive years to find out the characteristics of the gonadal cycle and the possible changes that occur during the different seasons.

MATERIALS AND METHODS

Monthly collections of adult land snails *Scutalus tupacii* were carried out from September 1988 to March 1991 in "Reserva Aguas Chiquitas" (El Cadillal, Tucumán, Argentina), which is part of the subtropical tucuman-bolivian forest. The samples were divided into two groups. One group used for histological studies was fixed in Bouin's or in Baker's fixative (Humanson, 1979). A second group was used for anatomical analysis, after being relaxed in cooled boiled water. These were then immersed in Baker's fixative. The ovotestis and hermaphroditic duct were dissected out, dehydrated in an ascending alcohol series, embedded in Paraplast and sectioned at 6 µm. Sections were stained with Ehrlich haematoxylin-eosin and Mallory (Azan) Heidenhain (Humanson, 1979).

Monthly, ovotestis and hermaphroditic ducts were fixed in Karnovsky's solution (Karnovsky, 1965) with 0.1 M phosphate buffer (pH 7.2), post-fixed in 2% osmium tetroxide and embedded in Epon-araldite. Semi-thin sections of 1 µm were stained with toluidine blue and examined by light microscope. Voucher specimens have been deposited in the Fundación Miguel Lillo's malacological collection (Catalogue No. FML 001000).

RESULTS

As in other pulmonate gastropods, the ovotestis or hermaphroditic gonad of adult *Scutalus tupacii* is found embedded in the digestive gland which is located in the upper whorls of the shell. The activity and the size of the ovotestis depend upon the age and size of the animal as

well as the season. The structure of the ovotestis was found to be similar to most pulmonates (Joosse and Reitz, 1969; Luchtel, 1972a, b; de Jong-Brink *et al.*, 1977).

From November to May (end of Spring to early Autumn), the acini are whitish and clearly separated from each other. At the beginning of April, a progressive reduction in the size of the ovotestis occurs that peaks between June and July (Winter). The decrease in length and diameter of the acini are accompanied by a change in colouration from white to light brown. The pigmentation is more evident in the distal part of the acini. In October the ovotestis progressively begins to recover its size and color.

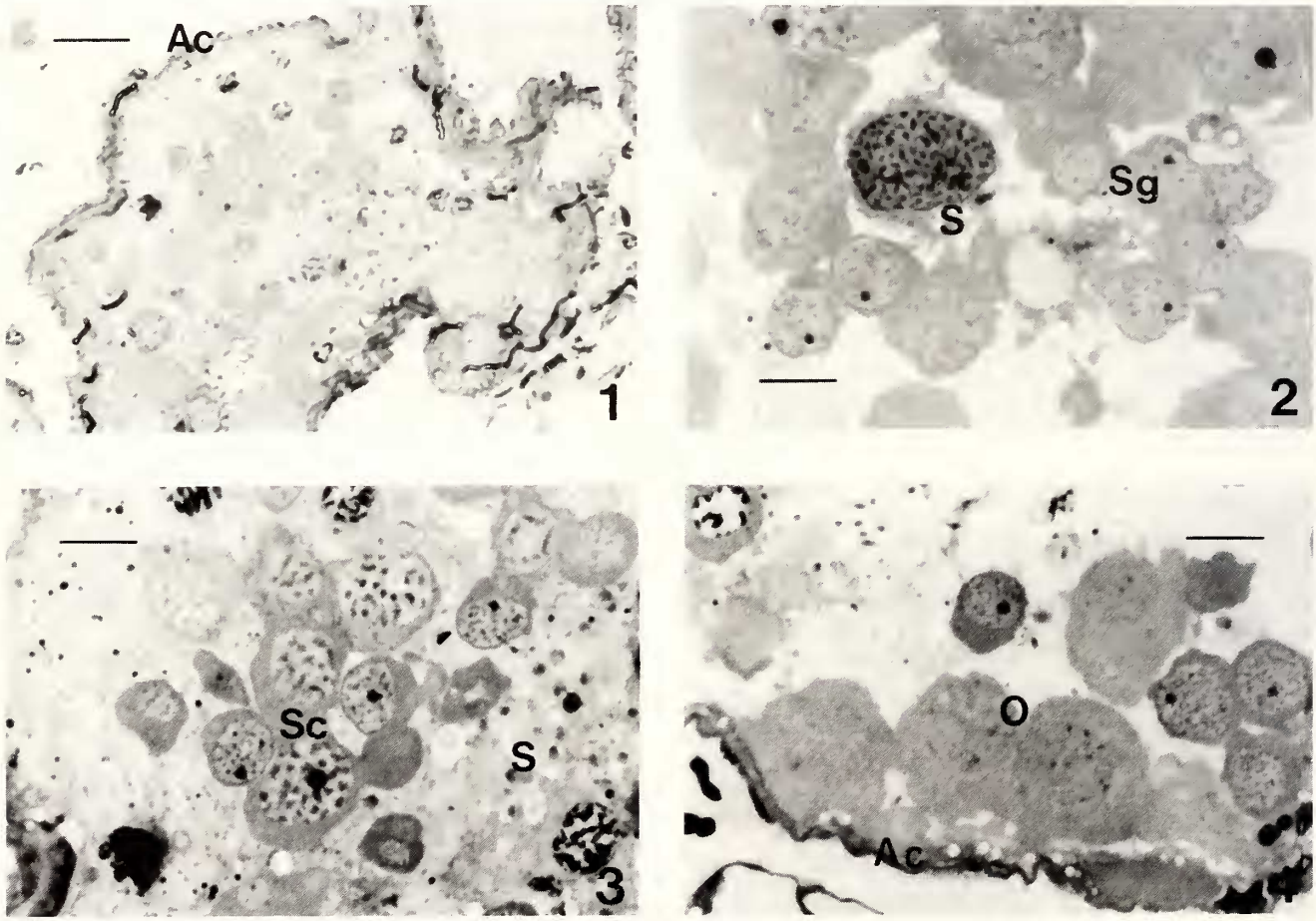
The annual cycle of the ovotestis can be divided into three phases of activity: 1) Post-breeding phase (March-September), Fall-Winter; 2) Pre-breeding phase (September-October), Spring; 3) Breeding phase (November-February), Spring-Summer.

1) Post-breeding phase (Figs. 1-4):

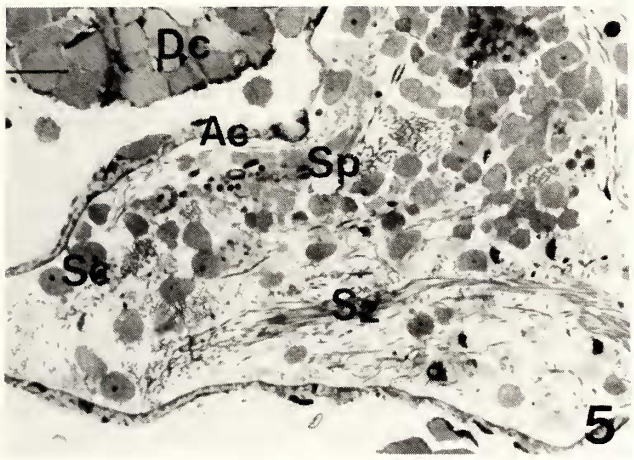
During this phase the acini are small in diameter with abundant interacinar space (Fig. 1). The post-breeding part of the cycle is characterized by an abundance of spermatogonia and primary spermatocyte (Figs. 2, 3). Oogonia are present isolated or in clusters of two to four cells and are always located inside the epithelium lining the acini, remaining in contact with the basal lamina. These cells are recognizable by a poorly contrasted cytoplasm (Fig. 4). Spermatids are scarce and are present only in early stages of differentiation. Few morphologically mature spermatozoa are present in the lumen of the acini. In the distal region of the acini the presence of mature Sertoli cells is remarkable. Their abundant cytoplasm is highly vacuolated and extends into the lumen, contributing to the "packed' appearance of the acini. Male germ cells at different stages of differentiation are clearly embedded within the same Sertoli cell.

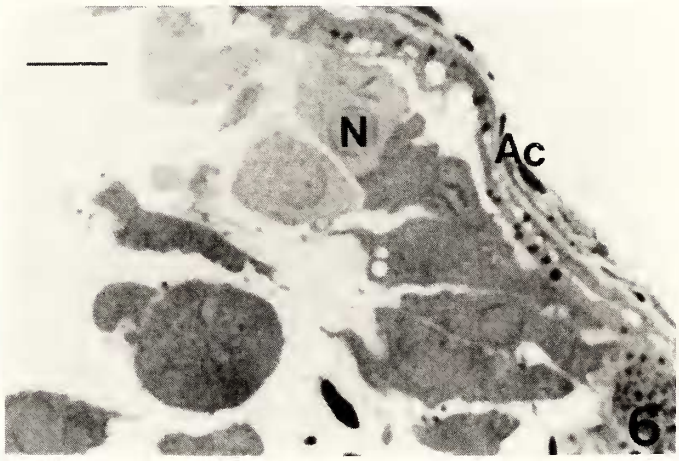
2) Pre-breeding period (Figs. 5-10):

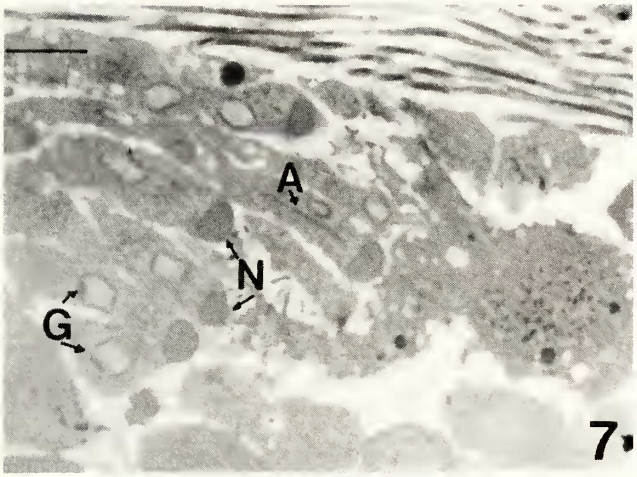
Externally the ovotestis maintains the same appearance as in winter. The acini have few or lack spermatozoa

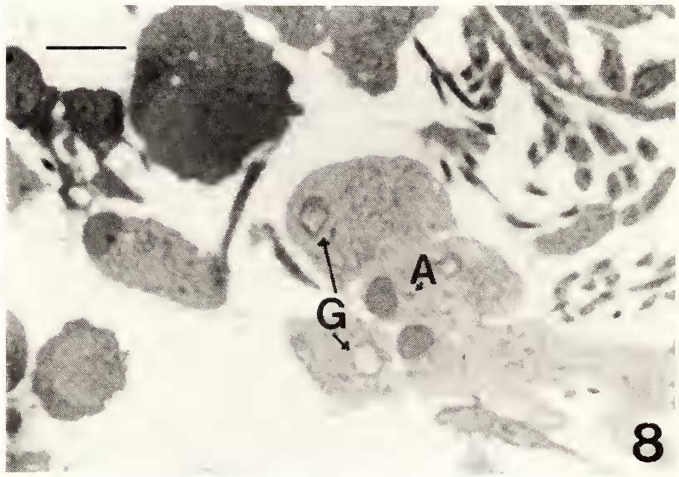


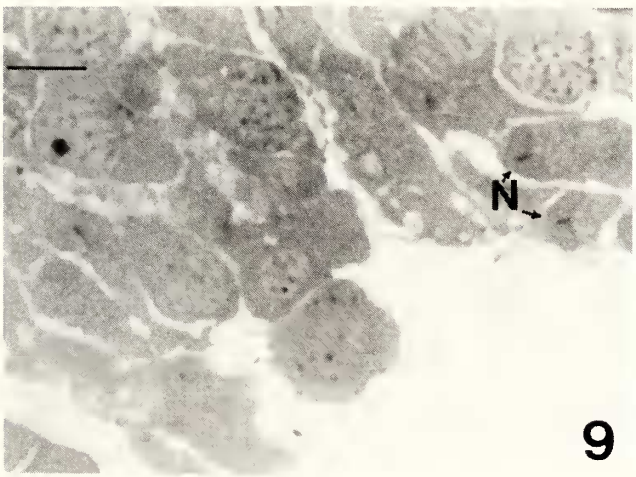
Figs. 1-4: Post-breeding period. **Fig. 1.** General appearance of the acini during the Post-breeding period (Ac, acinar wall; scale bar = 20 μm). **Fig. 2.** Group of spermatogonia (Sg) in close contact with a Sertoli cell (S) with dark nuclei (scale bar = 10 μm). **Fig. 3.** Group of spermatocytes (Sc) embedded in the cytoplasm of a Sertoli cell (S). (scale bar = 10 μm). **Fig. 4.** Three young oocytes in contact with the acinar wall (O, oocyte; Ac; acinar wall, scale bar = 10 μm).

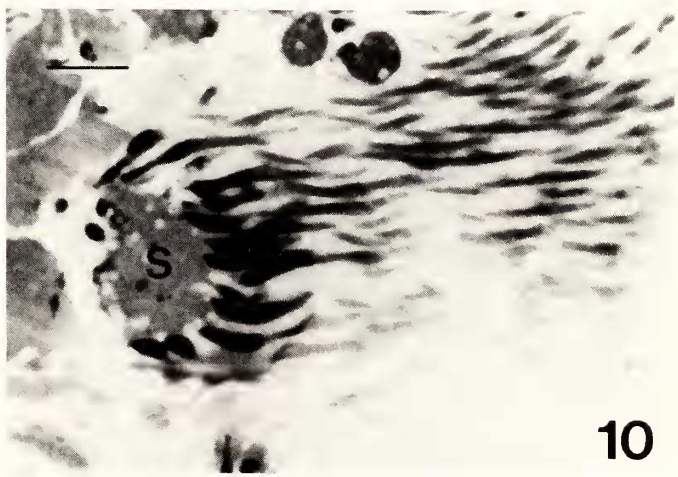












Figs. 5-10: Pre-breeding period. **Fig. 5.** General aspect of an acinus during the pre-breeding period (Ac, acinar wall; Sc, spermatocytes; Sz, spermatozoa; Sp, spermatids; S, Sertoli cells; scale bar = 40 μm). **Figs. 6-9.** Different stages of spermatid maturation. **Fig. 6.** Early spermatid stage. These cells possess a round nucleus with anterior and posterior plaque already differentiated (N, nucleus; scale bar = 10 μm). **Figs. 7-9.** Mid spermatid stage. The nucleus becomes indented at the posterior plaque. An elongating axonome is visible. (N, nucleus; A. axoneme; G, Golgi apparatus; scale bar = 10 μm). **Fig. 10.** Late spermatid stage. Elongated nucleus with condensed chromatin. The heads are embedded in the cytoplasm of a Sertoli cell (S). (scale bar = 10 μm).

(Fig. 5). Toward the end of September and the beginning of October spermatids proliferate and different stages start to become apparent (Figs. 6-10). Spermatids become more abundant than spermatocytes. Also a growing number of spermatozoa can be observed in the lumen of the acinus. At the bottom of the acini vitellogenic oocytes are present but never more than one per acinus.

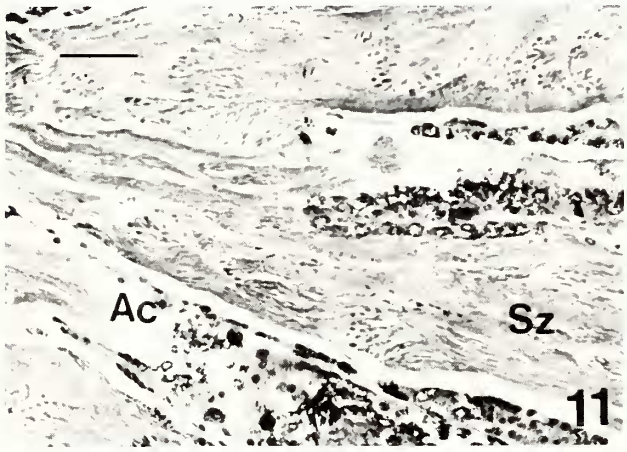
3) Breeding period (Figs. 11-14):

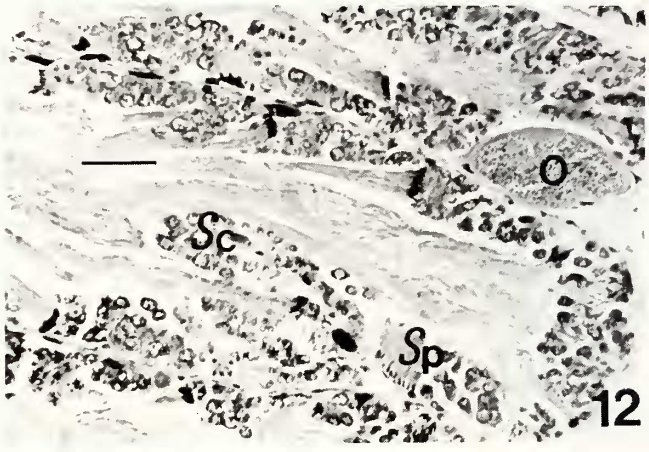
During this part of the cycle all the spermatogenic stages, from spermatogonia to spermatozoa are present in the ovotestis (Figs. 11, 12). The lumen of the acini are filled with spermatozoa. Concerning the female line, previtellogenic and vitellogenic oocytes (Figs. 13, 14) (Griffond and Bolzoni-Sungur, 1986) are distributed along the acini with a tendency towards maturation near the bottom of the acinus.

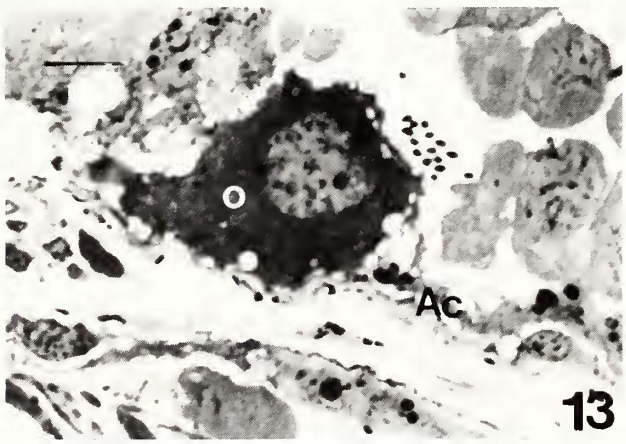
In longitudinal sections of ovotestis (Figs. 11, 12), no interacinar space is observed and the layers of the adjacent acini are in contact. The ovotestis of individuals that had just copulated were almost empty, lacking mature male gametes.

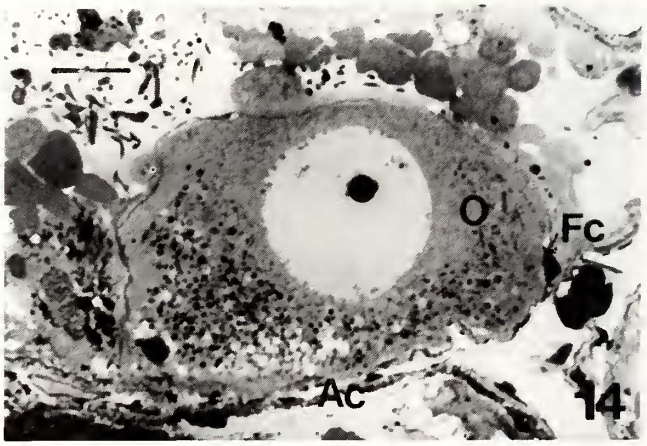
The male phase in the ovotestis of *Scutalus tupacii* starts during the post-breeding period with an active proliferation of spermatogonia and spermatocytes, coincident with hibernation (end of autumn and during winter). Although mature spermatozoa are present in the ovotestis in every month of the year it is important to remark that spermiogenesis is inactive during winter. Therefore the mature gametes are less abundant in the acini while the snails are hibernating. As soon as spermiogenesis begins, toward the end of October and beginning of November, the lumen of the acini fills with spermatozoa.

Mature oocytes are present throughout the year being especially abundant during the breeding period.









Figs. 11-14: Breeding-period. **Fig. 11.** Longitudinal section of the proximal part of several acini. The lumens are saturated with spermatozoa (Sz) (Ac, acinar wall; scale bar = 40 μm). **Fig. 12.** Longitudinal section of the distal portion of several acini. The more advanced stages, spermatocytes (Sc), spermatids (Sp) and mature oocytes (O) are observed in this region (scale bar - 50 μm). **Fig. 13.** Young premeiotic oocyte resting on the acinar wall (Ac) (O, oocyte; scale bar = 10 μm). **Fig. 14.** Vitellogenic oocyte. Note the presence of follicular cells (Fc) that envelope the oocyte (O, oocyte; Ac, acinar wall; scale bar = 20 μm).

Female cell proliferation begins in autumn, during May and June, but the number of mature oocytes grow between November and December in the pre-breeding and breeding periods.

The seminal vesicle stores spermatozoa throughout the year. In the summer months, however, a noticeable increase in both the number of stored male gametes and the diameter of the hermaphroditic duct was noted. Although rather constant patterns were recorded in the three reproductive periods, individual variation in the lengths of the periods existed.

SEASONALITY IN THE REPRODUCTION

According to W. Koppen's climatic classification (Torres Bruchman, 1976), the region that this population inhabits has a mesothermal climate with a dry winter (temperature of the warmest month higher than 22°C and of the coldest month lower than 18°C). The vegetation corresponds to a basal subtropical forest which is characterized by the presence of big trees [*Phoebe porphyria* (Grisebach) Mez, *Jacaranda mimosifolia* D. Don, *Juglans australis* Grisebach and *Tipuana tipu* (Benth.) O. Kuntze] associated with epiphyt plants like *Phlebodium aureum* (L.). Abundant ferns with wide front fronds cover the ground. The presence of a wet season in the region is the key to understand the pattern of activity of these land snails. The length of this period is approximately 6 months with the heaviest rains between January and March (Fig. 15). Winter is the dry season when the snails are most of the time dormant. In1989, the dry season was longer than in 1990 and consequently the hibernation period was prolonged. At the beginning of the dry period most of the animals bury themselves vertically with the aperture of the body whorl below the soil and the spire above. Other snails simply protect themselves under a thick layer of dry leaves and secrete a thin, transparent epiphragm. No mucus tracks were observed during these months (temperature range: 0 - 20°C). Another noticeable change is the retraction of the animal's body into the shell as a consequence of weight and water loss.

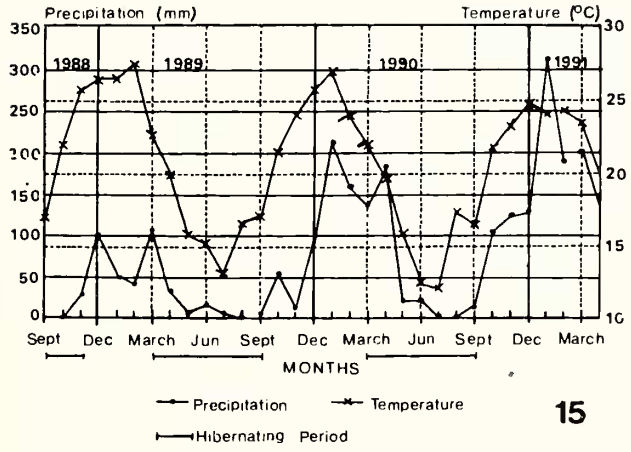


Fig. 15. Dry and wet seasons from 1989 to 1991.

Reproductive activity begins between October and November when the first rains can deeply moisten the soil. From this moment it is possible to observe copulating snails, especially nocturnally, but also during certain days, in particular the rainy ones. Mating can last several hours. The first clutch of eggs, both in 1988 and 1989, were found in November, generally buried in moist soil at the base of large trees. The eggs have an opaque-white albuminous substance within a nearly transparent membrane. The number of eggs per clutch ranged from 70 to 120 with diameters from 2.5 to 3.0 mm. Under laboratory conditions the duration of development ranged from 15 to 18 days.

DISCUSSION

Three different regions in gonadal acini of *Helix aspersa* juveniles (Griffond and Bride, 1987) and in *Biomphalaria glabrata* (Say) (de Jong Brink, 1976) have been described. In adult *Scutalus tupacii* these regions are not clearly identifiable. A trend toward a compartmentalization in male medullar and female cortical regions occurs. Also there is a higher frequency of vitellogenic oocytes, mature follicular and Sertoli cells at the bottom of the acinus. Morphologically mature spermatozoa are found in the acinar lumen with their nuclei oriented toward the bottom and the flagellae toward the neck of the acinus.

Despite the lack of strict regionalization, most of the mature gametes are typically found at the bottom of each acinus. Young germinal cells are distributed randomly along the acini. Coincidently, Runham and Hogg (1979) have found in *Deroceras reticulatus* (Müller) that there is a gradient of oocyte size being largest at the acinar base. However, they also noted that the great enlargement of the acini could, by itself, completely explain the observed oocyte distribution: largest oocytes arise first from the base, the oldest part of the acinus and remain there without any active movement.

Although proliferation of male and female germinal cells is practically coincident, male gametes reach maturity at least one month before the female ones. A number of studies, carried out in several species (Lusis, 1966; Runham and Laryea, 1968; Luchtel, 1972; Runham, 1978) support the hypothesis of a clear separation in time of male and female phases without superposition in the time of proliferation of the germinal cells. During the breeding period of *Scutalus tupacii* both mature spermatozoa and oocytes are present in the ovotestis, coincidently with the summer

months and wet season. During winter, the dry season, hibernation takes place and gonadal activity is minimal with predominating male and female juvenile germinal cells.

Luchtel (1972) suggests that *Arion circumscriptus* L. would be defined as a protandrous hermaphrodite considering the time at which the male and female gametes reach maturity. However, in terms of time of differentiation of the spermatogonia and oogonia the species could be considered a simultaneous hermaphrodite and finally in terms of appearance of primary spermatocytes and oocytes *Arion* could be a protogynous hermaphrodite.

Scutalus tupacii could be considered a protandrous hermaphrodite if we define "protandry" as the maturation of male gametes earlier than the female. However the presence of mature oocytes and spermatozoa is coincident during the breeding period without a great separation in time of both phases. Among the pulmonates already studied, most are true protandric hermaphrodites (Lusis, 1966; Smith, 1966; Runham and Hunter, 1970; Parivar, 1978) except for the Achatinellidae which are registered as protogynous.

Based upon the analysis of the climatic data (Fig. 15) and mainly upon observations in the field, the annual cycle of activity in *Scutalus tupacii* depends principally upon precipitation. With the beginning of the wet season in the summer the snails start their reproductive activity, copulating and laying eggs during these months. The duration of hibernation is intimately related to the duration of the dry season. The other factors such as the temperature and photoperiod, are not as important as precipitation and humidity of the habitat in the actvitity cycle of *S. tupacii.*

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